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小红参的抗癌环己肽配糖体*

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摘要 从小红参(*Rubia yunnanensis*)中分离得到 1 个具有抗癌活性的环己肽配糖体新成分 RY-Ⅰ，经化学及光谱学方法确定了其结构，证明此化合物由 2 个 L-丙氨酸、1 个 D-丙氨酸、3 个 N-甲基-L-酪氨酸 6 个氨基酸经肽键缩合与 1 个葡萄糖构成的环己肽配糖体，6 个氨基酸缩合形成十八员环，其中两个酪氨酸之间的苯环经氧桥连接又形成 1 个具有较大张力的十四员环。活性测试表明 RY--Ⅰ具有抗癌活性。此外还分离得到 RY-Ⅰ的甙元 RA-Ⅴ，也是 1 个有抗癌活性的成分。

关键词 小红参; 茜草科; 抗癌活性; 环肽配糖体; RY-Ⅰ

ANTITUMOR GLYCOCYCLOHEXAPEPTIDE FROM RUBIA YUNNANENSIS

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Abstract A new antitumor glycocyclohexapeptide, RY-Ⅰ, was isolated from *Rubia yunnanensis* together with its aglycone, RA-Ⅴ, which is an antitumor compound. The structure of RY-Ⅰ was elucidated by means of chemical and spectral methods and its biological activity was studied.

Key words *Rubia yunnanensis*; Rubiaceae; Antitumor; Cyclopeptidic glycoside; RY-Ⅰ

INTRODUCTION

In the late 1970's some interesting antitumor cyclohexapeptides were isolated from plants of the genera *Bouvardia* [1] and *Rubia* [2], thus made the plants possess a new potential application and stimulated further chemical studies on these plants.

Rubia yunnanensis Diels is a species native to Yunnan, China and it can be used as substitute material of *R. cordifolia* [3] which is a well-known Chinese traditional medicine. Also the roots of *R. yunnanensis* Diels, known as "Xiao-Hong-Sheng", are used as antitumor drug in Yunnan. As a search for new antitumor drugs, the chemical constituents of roots of *Rubia yunnanensis* were studied. Recently we reported 3 arborane type triterpenoids isolated from the plant [4,5]. In this paper we describe the isolation and structure elucidation of a new antitumor cyclohexapeptide, RY-Ⅰ, which is the first glycosidic one of the type found in nature together with its aglycone which is also an antitumor compound. Biological activity of RY-Ⅰ is reported as well.

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RESULTS AND DISCUSSION

An ethanolic extract of *Rubia yunnanensis* was subjected to column chromatography on activated charcoal to yield several fractions. These fractions were further chromatographed repeatedly on silica gel and then silanized silica gel. Compound 2 was identified as deoxybouvardin (RA−V) by comparison of physical and spectral data with those of literature [6,7].

Compound 1 showed negative reaction when tested with ninhydrin but after hydrolyzed with concentrated HCl the result was opposite, suggesting the presence of a cyclic peptide chain in the molecule. In the ^{1}H NMR spectrum, three methyl groups adjoining to methine at δ1.08(3H, d, J=6.7Hz), 1.23(3H, d, J=7.0Hz), and 1.28(3H, d, J=6.9Hz), three N−methyl groups at δ2.61(3H, s), 2.90(3H, s), and 3.05(3H, s), a O−methyl group at δ3.76(3H, s), protons of a sugar at δ3.0—5.5 among which the anomeric signal is at δ4.98(1H, d, J=7.6Hz), and aromatic protons at δ6.6—7.8 were observed. The high coupling constant value is consistent only with the β configuration for the anomeric linkage.

Also, the carbon signals due to three C− methyl, three methylene, three N−methyl, an O−methyl, six methine, six carbonyl, eighteen aromatic (eleven tertiary and seven quarternarycarbons) and six sugar carbons which were easily identified appeared in the ^{13}C NMR DEPT spectrum(Table 1).

Above spectral data are very close to those of cyclohexapeptides isolated from other *Rubia* species [6,7] indicating that RY−Ⅰ is also composed of two L−alanines, a D−alanine, and three modified N−methyl−L−tyrosines if the sugar signals were not taken into account temporarily. The ^{13}C NMR (DEPT) spectrum showed the sugar signals at δ102.9(CH, anomeric), 74.9(CH), 78.2(CH), 71.4(CH), 77.9(CH), and 62.6(CH_2), which were coincident with a glycosidic glucose, indicating that the compound was a glucoside with a cyclohexapeptide as aglycone.

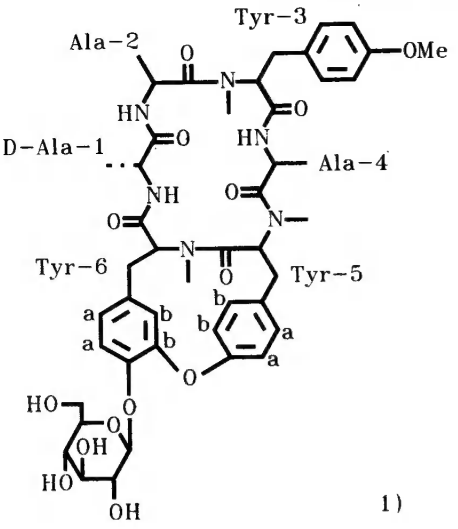
Two positions could be considered possible to link the glucose on the aglycone and one was Tyr−3ζ while the other Tyr−6ζ. Usually this kind of problems can be solved by comparing ^{13}C NMR spectra of the glycoside with those of its possible aglycones but in this case the results were still ambiguous since the glycoside could not be solved in chloroform in which the NMR spectra of the possible aglycones were measured reportedly [6,7]. Thus the comparison would be affected by solvent effects inevitably. FAB−MS of RY−Ⅰ (Scheme 1) excluded the former alternative mentioned above and this was confirmed by hydrolysis of RY−Ⅰ with 1mol/L HCl which afforded glucose and cyclohexapeptide identical with deoxybouvadin or RA−V by comparing its spectral data with literature [6,7].

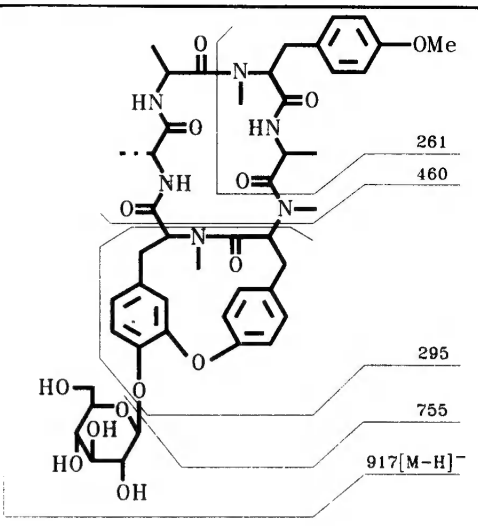
The results of antitumor activity of RY−Ⅰ on P−388 leukemia are shown in table 2.

EXPERIMENTAL

Plant material. Roots of *R. yunnnanensis* were collected from Yunnan,China. Mps: uncorr. ^{1}H and ^{13}C NMR with TMS as int. standard. Silica gel G (10−40, Qingdao Marine Chemical factory, China) and silanized silica gel (LiChroprep RP−8, 40−63 μm, Merck) were used for CC. All solvent systems for chromatography were homogeneous.

Extraction and separation. The ground roots of *Rubia yunnanensis* (10.0kg) were extracted with MeOH (3×15l) and then concentrated to dryness in vacuo. 1.0kg extract was obtained. The extract was subject to activatedcharcoal chromatography and gradient eluted with $CHCl_3$, EtoAc and $CHCl_3$−MeOH (v/v, 3/2). These fractions afforded 21 compounds including RY−Ⅰ (1, 163mg) and deoxybouvardin (RA−V) (2, 171mg) in total after chromatographed repeatedly with silica gel or silanized silica gel. Both





Scheme 1. Formation of fragments in RY−I upon FAB−MS

Table 1. ^{13}C NMR chemical shifts of RY−Ⅰ(1) and RA−V(2)

Carbon	1	2	Carbon	1	2
Ala−2β	16.43	16.31	Tyr−6γ	131.63	127.45
Ala−4β	18.84	18.33	Tyr−3δ	131.46	130.08
Ala−1β	21.05	20.60	Tyr−3γ	132.31	130.47
Tyr−6N−Me	30.49	29.34	Tyr−5δa	131.90	130.91
Tyr−5N−Me	31.09	30.45	Tyr−5δb	134.08	132.87
Tyr−3β	33.71	32.79	Tyr−5γ	136.93	135.48
Tyr−6β	36.63	35.53	Tyr−6ζ	145.40	142.99
Tyr−5β	37.40	36.75	Tyr−6εb	154.87	51.15
Tyr−3N−Me	40.33	39.79	Tyr−5ζ	159.73	157.92
Ala−2α	45.56	44.50	Tyr−3ζ	160.01	158.32
Ala−4α	47.80	46.33	Tyr−6CO	170.99	168.14
Ala−1α	48.57	47.81	Tyr−5CO	171.29	169.09
Tyr−5α	55.69	54.27	Tyr−3CO	172.29	170.61
Tyr−3O−Me	55.69	55.17	Ala−4CO	172.98	171.92
Tyr−6α	58.69	57.36	Ala−1CO	173.40	172.28
Tyr−3α	68.74	68.32	Ala−2CO	174.56	172.72
Tyr−6δb	115.04	113.10	Glc−1	102.82	
Tyr−3ε	115.81	113.97	2	74.84	
Tyr−6εa	118.63	115.91	3	78.06	
Tyr−6δa	122.64	121.52	4	71.29	
Tyr−5εb	125.12	124.09	5	77.80	
Tyr−5εa	127.28	125.84	6	62.59	

Ala−1 = D−alanyl; Ala−2 = Ala−4 = L−alanyl; Tyr−3 = Tyr−5 = Tyr−6 = L−tyrosyl.

Table 2 Antitumor effects of RY−Ⅰ on P−388 leukemia

Sample concentration (μg / mL)	Inhibition rate(%)
100	100
10	80
1	28

1 and 2 were heated with ninhydrin and no colour change was observed. After hydrolysed in sealed tubes at 120 ° with concentrated HCl for a night 1 and 2 showed red spots when heated with ninhydrin on filter paper.

RY－Ⅰ (1). powder, mp 226–228 ° $[\alpha]_D^{25}$–267 ° (MeOH, c 0. 26) . FABMS(negative ion mode) m / z 917[M－H]⁻. ^{13}C NMR: (Table 1). ^1H NMR(400 MHz, CD_3OD): 1.08(3H, d, J＝6.7Hz, Ala－4β), 1.23[3H, d, J＝7.0Hz, Ala－2β], 1.28(3H, d, J＝6.9Hz, D－ala－1α), 2.61(3H, s, Tyr－6N－Me), 2.90(3H, s, Tyr－3N－Me), 3.05(3H, s, Tyr－5N－Me), 3.76[3H, s, Tyr－3O－Me), 4.98(1H, d, J＝ 7. 6Hz,anomeric H of Glc).

Acid hydrolysis. RY－Ⅰ (20mg) in MeOH－H_2O (v / v, 1 / 1)was refluxed with 1mol / L HCl for 7h. After neutralized with Ag_2CO_3 the mixture was extracted with $CHCl_3$. Evapn of the extract game the aglycone 2 (11mg) which was identical with natural deoxybouvardin by mp and TLC comparison with authentic sample. PC (paper Xinhua, ascending mode, solvent: *n*－BuOH－AcOH－H_2O, 4 : 1 : 5, upper phase) of the residue indicated the presence of glucose.

Compound 2 was identified as deoxybouvardin (RA－Ⅴ) by comparing its NMRspectral data with those of literature [6, 7] .

Antitumor effect of RY－Ⅰ. DMSO was used as a solubilizing agent, and the concentration of sample solution was 100 μg / mL, 10 μg / mL, and 1μg / mL respectively. The inhibition rates of sample against P－ 388 cells are shown in table 2. P－388 cells were inoculated:180 μL cell solution of (2.5–3)× 10^5cells / mL was added into each hole, these cell solution were laid aside for 30min., and then 20 μL sample solution was added into each hole and reacting for 24h. Cells were counted by trypan blue colouring method. Contrast groups were cultured as the the same as above.

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REFERENCES

[1] Jolad S D, Hoffmann J J, Torrance S J et al. Bouvardin and deoxybouvardin, antitumor cyclic hexapeptides from Bouvardia ternifolia (Rubiaceae). *J Am Chem Soc* 1977; **99**: 8040

[2] Itokawa H, Takeya K, Mihara K et al. Studies on the antitumor cyclic hexapeptides obtained from Rubiae radix. *Chem Pharm Bull.* 1983; **31**: 1424

[3] Wang Shuxian, Hua Huiming, Wu Lijun. Development of Chinese traditional medicine Qiancao. Shenyang Yao－xueyuan Xuebao 1990; **7**: 303

[4] Zou Cheng, Hao Xiaojiang, Chen Changxiang et al. A new antitumor glycocyclohexapeptide and arborane type new triterpenoids from Rubia yunnanensis. Acta Botanica Yunnanica 1992; **14**(1): 114

[5] Zou Cheng, Hao Xiaojiang, Chen Changxiang et al. New arborane type triterpenoids from Rubia yunnanensis (Ⅰ) Acta Botanica Yunnanica 1993; **15**(1): 89

[6] Bates R B, Cole J R, Hoffmann J J et al. Solution forms of bouvardin and relatives from NMR studies. 6－O－methylbouvardin. *J Am Chem Soc* 1983; **105**: 1343

[7] Itokawa H, Takeya K, Mori N et al. Isolation and antitumor activity of cyclic hexapeptides isolated from Rubiae radix. *Chem Pharm Bull.* 1984; **32**: 284